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Brenda J. Little, Patricia A. Wagner, Kevin R. Hart, and Richard I. Ray

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SPATIAL RELATIONSHIPS BETWEEN BACTERIA AND LOCALIZED CORROSION

Brenda J. Little, Patricia A. Wagner, Kevin R. Hart, and Richard I. Ray
Naval Research Laboratory
Stennis Space Center, MS 39529-5004

ABSTRACT

Spatial relationships between bacteria and polarization were examined using microbiological and surface analytical techniques. Corrosion products produced by well-established artificial crevices in 304 stainless steel in abiotic seawater were associated with large numbers of bacteria after brief exposures to natural seawater. The presence of bacteria did not alter the distribution or composition of the corrosion products. Cathodic polarization increased the number of viable marine bacteria and extracellular debris on 304 stainless steel. Bacterial colonization and metabolism can fix anodes and cathodes; however, abiotic polarization can influence the number and types of bacteria associated with the surface. Spatial relationships between bacteria and localized corrosion cannot independently be interpreted as causal.

Keywords: microbiologically influenced corrosion, cathodic polarization, crevice corrosion

INTRODUCTION

Microbiologically influenced corrosion (MIC) does not produce any unique form of localized corrosion. Instead, MIC can result in pitting, crevice corrosion, and under-deposit corrosion, in addition to enhanced galvanic and erosion corrosion. Since there are no definitive tests for MIC, diagnosis requires a combination of electrochemical, surface analytical, and microbiological techniques. Often the most convincing evidence for MIC is the spatial relationship that exists between specific physiological types of bacteria and the manifestation of localized corrosion. Spatial relationships are usually quantified using microbiological culture techniques or microscopy. For example, Little et al.[1] demonstrated sulfate-reducing bacteria (SRB) within sulfide corrosion products on copper alloys. Cells were heavily encrusted with copper sulfides. Similarly, iron-depositing bacteria have been identified in tubercles on stainless steels.[2] In oxygenated media, tubercles cause aggressive under-deposit corrosion in stainless steels by establishing a relatively small anode surrounded by a large cathode. In both cases, spatial relationships between bacteria and corrosion products were used to support the case for cause and effect, i.e., that microorganisms caused pitting. The role that bacteria play in producing/inducing/influencing anodic reactions has been documented. However, the impact that anodic and cathodic polarizations have on deposition or settlement of bacteria has not been adequately addressed. In this paper, experiments were designed to answer the following questions: (1) Do anodic and cathodic polarizations influence the distribution and type of bacterial cells on corroding surfaces?, and (2) Can spatial relationships between bacteria and localized corrosion be used to interpret MIC?

METHODS AND MATERIALS

Experiments were designed to insure that 304 stainless steel electrode surfaces had defined anodic or cathodic regions in abiotic media prior to exposure to bacteria. Natural Bay St. Louis, MS, seawater (25 ppt) to which an inoculum of *Oceanospirillum* sp. had been added was used as the source of bacteria for all biotic exposures. Open circuit potential measurements (E_{corr}) as a function of time were measured with Princeton EG&G potentiostats (Model 173, Model 273, and Model 350). Artificial crevices were initiated by placing a small piece of sterile wood under the gasket of the electrode holder across the face of the electrode. Identical electrodes were maintained without crevices. Electrodes were exposed to 700 ml filtered 35 ppt artificial seawater (0.10 μm Millipore filter) to allow the formation of corrosion products on specimens with crevices. After 11 days, 50 ml of natural seawater was added to the abiotic electrolytes containing electrodes with and without crevices. Abiotic controls were maintained for all exposures. After 5 days electrodes were removed, placed in seawater-buffered glutaraldehyde, and examined using environmental scanning electron microscopy (ESEM) coupled with energy dispersive x-ray analysis.

The effects of cathodic polarization on spatial distribution were examined by polarizing 304 stainless steel electrodes at −400 mV (vs. saturated calomel electrode (SCE)) for 72 hours and measuring E_{corr} for 4 additional days. Electrodes were polarized in natural Bay St. Louis, MS, seawater augmented with *Oceanospirillum* sp., and compared to unpolarized samples. Serial dilution cultures (MICKIT, Bioindustrial Technologies, Georgetown, TX) and ESEM were used to quantify bacterial groups on the surfaces of the unpolarized and polarized 1 cm^2 electrodes.

RESULTS

Corrosion products on 304 stainless steel in which an abiotic crevice was first induced were associated with bacteria after a 5-day exposure to natural seawater (Figure 1). Within the corrosion products, spirilla, rods, vibrio, and filamentous cells were identified (Figure 2a–e). Cell types tended to be isolated from one another and did not appear in consortia as one would expect if the surface had indeed been colonized by the marine bacteria. The remainder of the electrode surface was free of corrosion and only isolated bacterial cells could be located. Experiments were reproduced three times with the same result. In one case, large numbers of diatoms were associated with the corrosion products after the 5-day exposure to natural seawater (Figure 2f). In abiotic controls, corrosion products formed in an identical fashion (Figure 3a–d). In all cases, the corrosion products were enhanced in chromium relative to the base alloy. The presence of bacteria did not change the distribution or elemental composition of the corrosion products.

Cathodic polarization also influenced numbers and types of cells on the surface. Isolated cells could be found on unpolarized controls, whereas polarized samples were covered with small rods after the same 7-day exposure period (Figures 4, 5). In addition to cells, there was more extracellular debris on the polarized sample. Differences in cell numbers were substantiated with culture results. The natural seawater was positive for SRB (10^0–10^1), acid-producing bacteria (>10^4), facultative anaerobes (>10^4) and aerobic heterotrophs (10^3–10^4) cells per cc. The polarized 1 cm^2 surface was positive for all physiological types, while the unpolarized surface was negative for SRB and positive for other groups. Currents required during polarization decreased with time from approximately 20 to 4 μAcm^{-2}.

DISCUSSION

Because the diagnosis of MIC can be controversial, it is extremely important that the interpretation of spatial relationships be unambiguous. McNeil et al.[3] defined two subsets of microbiologically influenced sulfide attack of copper alloys—intermediated and induced—based, respectively, on whether or not SRB produced sulfides within a biofilm. In the two examples of MIC cited in the introduction, evidence that bacteria caused pitting corrosion was overwhelming. In the case of copper alloys, large numbers of SRB were cultured from the surface and sulfide minerals within the corrosion products were identified. In the absence of SRB, there would have been no corrosion. Similarly, iron-depositing bacteria produced the tubercle within which they were found. Tubercles created differential

aeration cells and under-deposit corrosion of the 316 stainless steel. Without the activities of iron-depositing bacteria there would have been no tubercles and no corrosion. Other surface deposits can result in under-deposit corrosion, and there are other mechanisms for formation of tubercle-like deposits. In experiments described in this paper, corrosion was well established before introduction of bacteria. Their presence in corrosion products cannot be used to interpret formation of the crevice. In fact, they are associated with the corrosion products and not directly with the pitting, as in the case of the iron-depositing bacteria. The exact spatial relationship in this case is subtle, but important. Dexter[4] made a similar observation with 316 stainless steel where he "suspected that the bacteria in the corrosion product mounds are not directly involved in corrosion initiation, but become associated with the corrosion site after initiation, perhaps because the electrochemistry creates a favorable environment for their growth." Franklin et al.[5] reported that bacterial cells were associated with anodic regions detected using scanning vibrating electrode microscopy. They were cautious not to interpret their data to indicate that the cells caused the anodic sites. They did observe that once bacteria were associated with anodic regions, those areas remained anodic for the duration of the experiments. Anodic sites not associated with bacteria were transient. Wagner et al.[6] observed that bacteria preferentially colonized fiber-reinforced composites along fibers or within scratches or breaks of fibers. They did not interpret the close spatial relationship of cells in association with fiber breaks as indicative that cells caused the fiber disruptions. Instead, they observed that fiber breaks were preferentially colonized.

Campaignolle et al.[7] and Licina et al.[8] independently developed techniques for measuring galvanic currents sustained by bacteria. Both techniques use cathodic polarizations and Licina et al.[8] stated that polarization "encouraged biofilm formation." Licina has not specifically addressed the differences in the microflora resulting from the polarization, only the necessity to polarize. He indicated that biofilms that formed on the anodes and cathodes of the Bi.George™ probe were different in cell numbers and morphologies (personal communication). Angell et al.,[9] using the concentric ring electrode approach,[7] demonstrated that in experiments where a galvanic current was maintained, the numbers of bacteria isolated from the anode were 10 to 100 times greater than numbers from the cathode. The authors speculate that the higher level of colonization could be the result of negatively charged bacteria being attracted to the positively charged anode. They did not report cell numbers for unpolarized electrodes. It is clear that bacteria would have settled on all surfaces over some period of time. It is not clear that the same numbers and types of cells would have settled in the absence of polarization. Angell et al.[9] specified that their "system does not seek to represent any natural occurring situation . . .". Nekoksa and Gutherman[10] and Guezennec et al.[11] showed that more marine bacteria settled on cathodically protected metals than on unpolarized ones. Gomez de Saravia et al.[12] and Videla et al.[13] demonstrated that cathodic polarization in a seawater medium increased numbers of SRB and decreased numbers of aerobic bacteria on carbon steel surfaces relative to unpolarized controls. They hypothesized that preferential increases or decreases in cell types were due to differences in cell isoelectric points.

CONCLUSIONS

It is not the purpose of this paper to refute or question the importance of spatial relationships in the interpretation of MIC, but to indicate that proper identification of MIC requires more data than simple cell numbers or pictures of bacterial cells in association with corrosion products. Corrosion products associated with crevices on 304 stainless steel were associated with large numbers of marine bacteria. Bacteria did not cause the crevice. Instead, the crevice was responsible for the location and concentration of bacterial cells in the area. The nature of the attraction/association has not been addressed in this paper. Cathodic polarization resulted in increased cell numbers on the surface of 304 stainless steel.

ACKNOWLEDGMENTS

REFERENCES

1. B. Little, P. Wagner, R. Ray, J. M. Jones, "Microbiologically Influenced Corrosion of Copper Alloys in Saline Waters Containing Sulfate-Reducing Bacteria," CORROSION/91, paper no. 101, (Houston, TX: NACE International, 1991).

2. G. Kobrin, "Reflections on Microbiologically Induced Corrosion of Stainless Steels," in Biologically Induced Corrosion, S. C. Dexter, ed., (Houston, TX: NACE International, 1986), p. 33.

3. M. B. McNeil, J. M. Jones, B. J. Little, Corrosion 47, 9(1991): p. 674.

4. S. C. Dexter, "Effect of Biofilms on Marine Corrosion of Passive Alloys," in Bioextraction and Biodeterioration of Metals, C. C. Gaylarde, H. A. Videla, eds., (Cambridge, UK: University Press, 1995), p. 129.

5. M. J. Franklin, D. C. White, H. S. Isaacs, "The Use of Current Density Mapping in the Study of Microbial Influenced Corrosion," CORROSION/90, paper no. 104, (Houston, TX: NACE International, 1990).

6. P. Wagner, R. Ray, K. Hart, B. Little, W. Bradley, P.-L. Chiou, "Microbial Degradation of Stressed Fiber Reinforced Polymeric Composites," CORROSION/95, paper no. 200, (Houston, TX: NACE International, 1995).

7. X. Campaignolle, J-S. Luo, J. Bullen, D. C. White, J. Guezennec, J.-L. Crolet, "Stabilization and Localized Corrosion of Carbon Steel by Sulfate-Reducing Bacteria," CORROSION/93, paper no. 302, (Houston, TX: NACE International, 1993).

8. G. Licina, G. Nekoksa, R. L. Howard, "An Electrochemical Method for On-Line Monitoring of Biofilm Activity In Cooling Water Using the BIoGEORGE™ Probe," in Microbiologically Influenced Corrosion Testing, J. Kearns, B. Little, eds., (Philadelphia, PA: ASTM, 1994), p. 118.

9. P. Angell, J.-S. Luo, D. C. White, Corrosion Science 37, 7(1995): p. 1085.

10. G. Nekoksa, B. Gutherman, "Cathodic Protection Criteria for Controlling Microbially Influenced Corrosion in Power Plants," EPRI NP-7312, Electric Power Research Institute, Palo Alto, CA, May 1991.

11. J. Guezennec, N. Dowling, M. Conte, E. Antoine, L. Fiksdal, "Cathodic Protection in Marine Sediments and the Aerated Seawater Column," in Microbially Influenced Corrosion and Biodeterioration, (Knoxville, TN, University of Tennessee, 1991), p. 6-43.

12. S. G. Gomez de Saravia, M. F. L. De Mele, H. A. Videla, "Interacciones de Biopeliculas Bacterianas y Compuestos Inorganicos Sobre Aceros Protegidos Catodicamente," 5th Congreso Ibero-americano de Corrosion y Proteccion, D. Grandosa, ed., (Tenerife, Islas Canarias, Spain: La Laguna, 1995), p. 201.

13. H. A. Videla, S. G. Gomez de Saravia, M. F. L. de Mele, "Early Stages of Bacterial Biofilm and Cathodic Protection Interactions in Marine Environments," 12th International Corrosion Congress, (Houston, TX: NACE International, 1993), p. 3687.
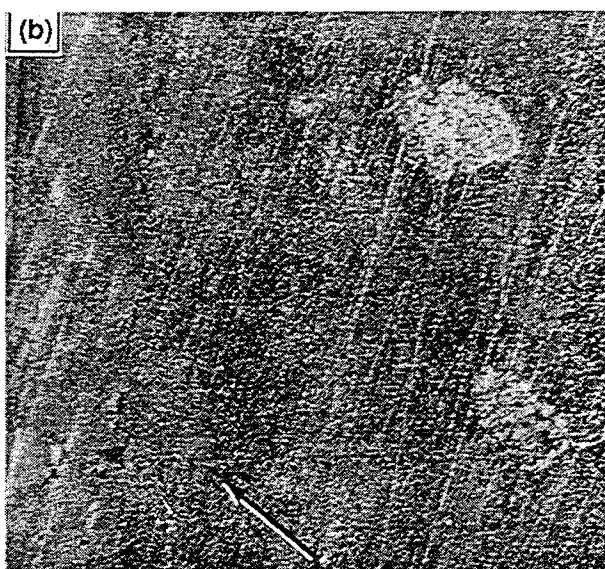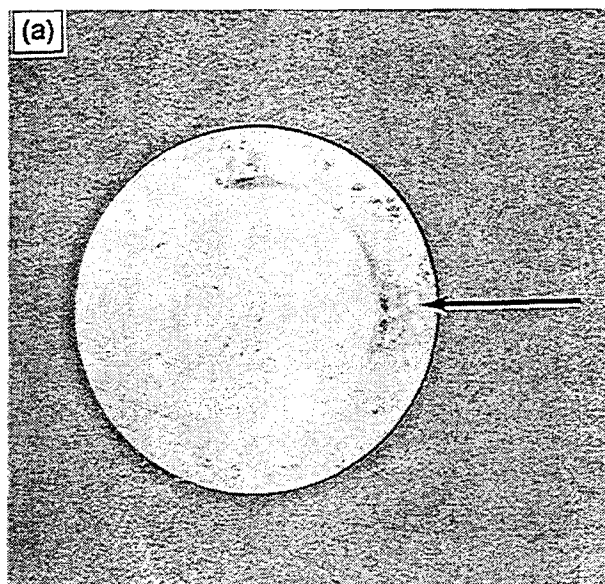
Figure 1. (a) Crevice in 304 stainless steel induced in abiotic seawater and then exposed to natural seawater for 5 days. Arrow indicates pits (3×) and (b) corrosion products (arrow) (30×).
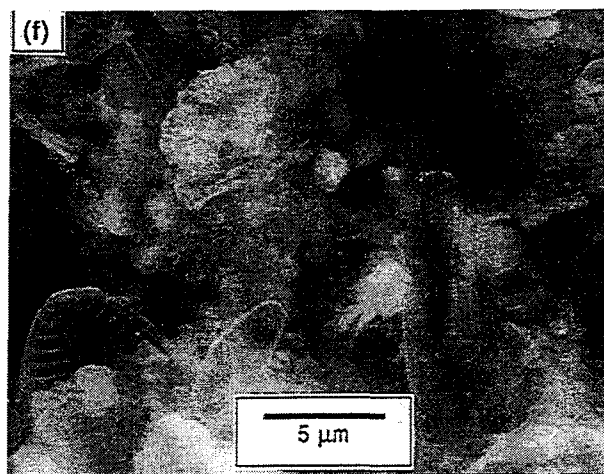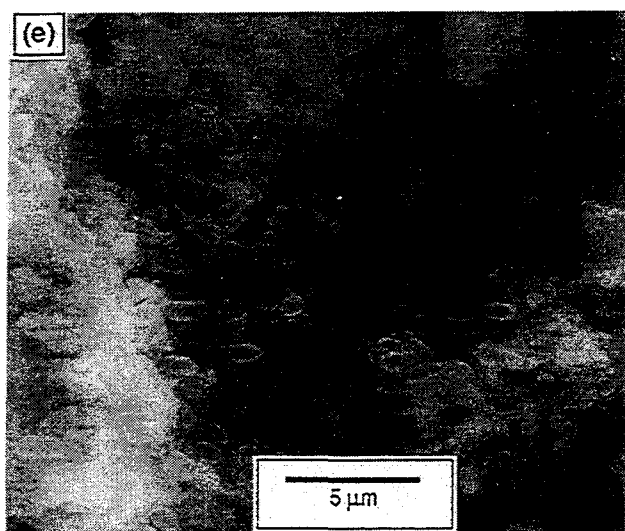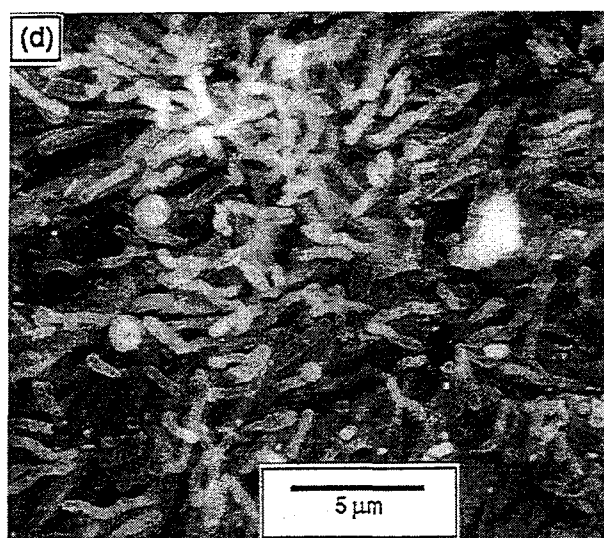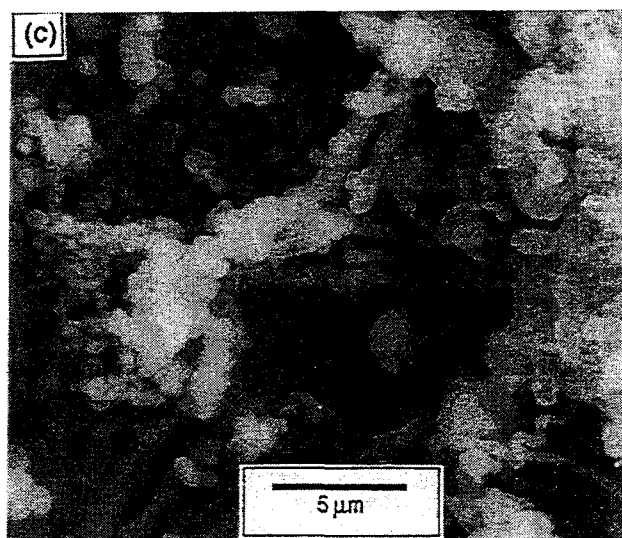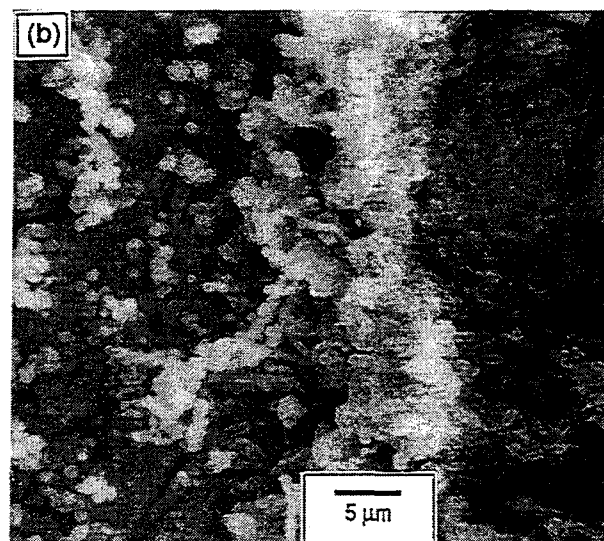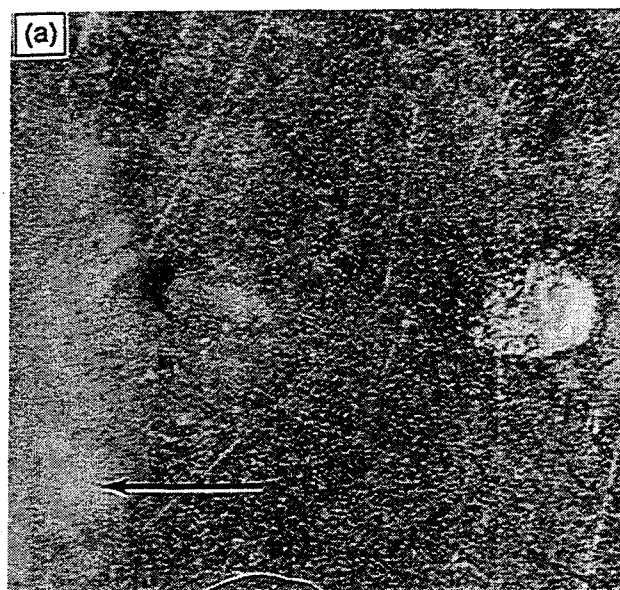
Figure 2. (a) Corrosion products (arrow) related to Fig. 1 (30×); (b, c) bacteria amassed within corrosion products, (d) spirilla and rod-shaped bacteria in association with corrosion products, (e) rod-shaped bacteria clumped together with corrosion products, and (f) diatoms associated with corrosion products.
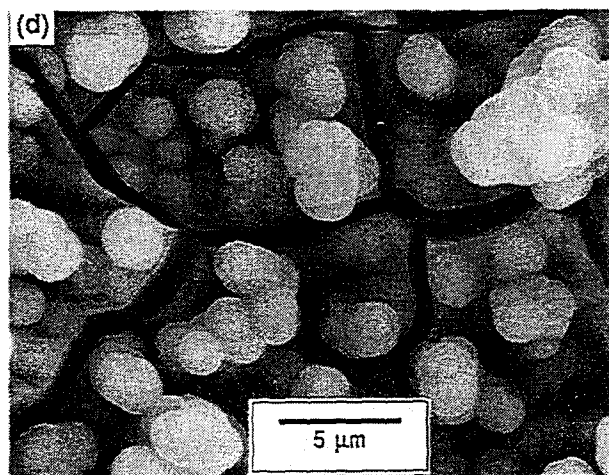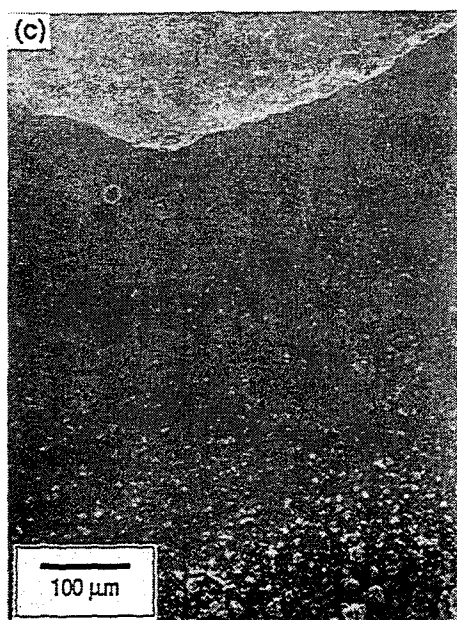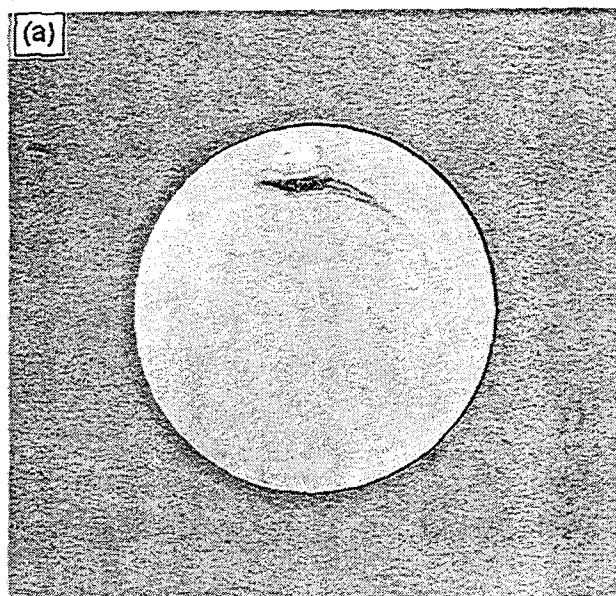
Figure 3. (a) Crevice in 304 stainless steel in abiotic seawater (3×), (b) corrosion products (arrow) (32×), (c) pit and associated corrosion products, and (d) corrosion products.
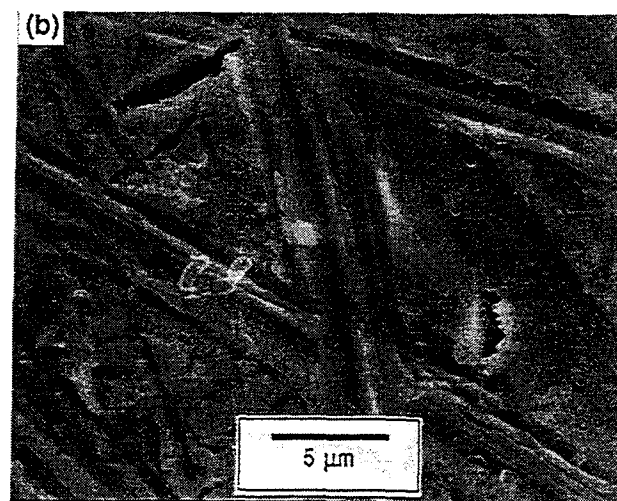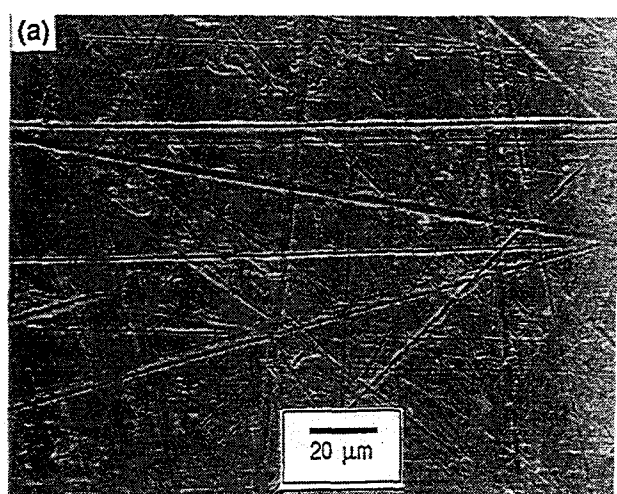
Figure 4. (a) Unpolarized 304 stainless steel surface and (b) isolated bacteria after 7-day exposure to natural seawater.
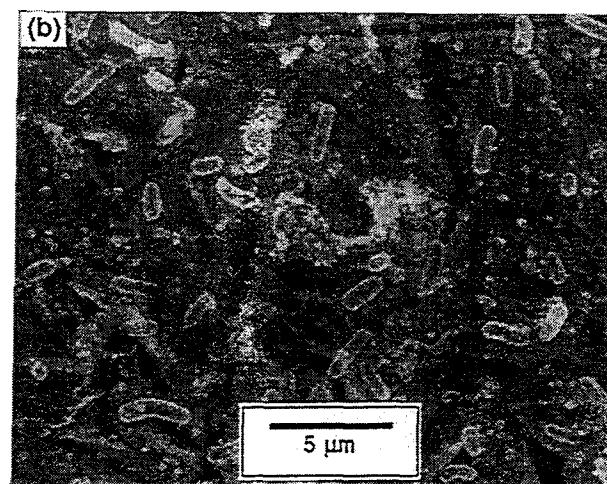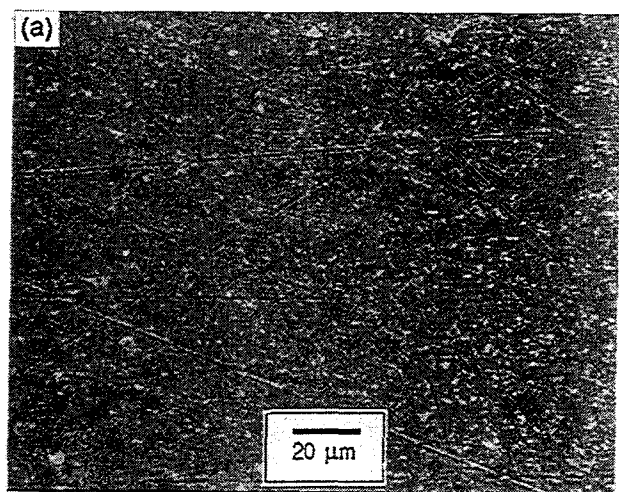

Figure 5. (a) Cathodically polarized 304 stainless steel (−400 mV SCE) and (b) bacteria and debris after 7-day exposure to natural seawater.